JOURNAL OF AGRICULTURAL RESEARCH

VOL. XI WASHINGTON, D. C., OCTOBER 8, 1917 NO. 2

ENDROT OF CRANBERRIES[1]

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INTRODUCTION

In the writer's early studies of diseases of the cranberry (*Oxycoccus macrocarpus*) occasional cultures of an undetermined organism of a rather striking and characteristic color appeared. These cultures, however, failed to produce spores, and their identity was not determined at the time. Since beginning cooperative experiments with the Massachusetts Agricultural Experiment Station on Cape Cod, this organism has been found to be the most frequent fungus causing a rot in Late Howe berries from the experimental plots and checks on the Massachusetts State Cranberry Experiment Station bog at East Wareham. The fungus has also been frequently found in diseased berries, especially the Late Howe, from Maine, New Jersey, Michigan, Wisconsin, Washington, and Oregon. Although this rot has been found thus far most abundantly in the Late Howe cranberry, further investigation may show that it is equally serious with some other varieties. It has been isolated from the following varieties: Bennett Jumbo, Cape Cod Beauty, Early Black, Early Ohio, Mathews, McFarlin, Perry Red, Prolific, Searles Jumbo, Selected Howe, Vose Pride, and several unknown varieties. It no doubt occurs in others that have not yet been examined. Sufficient observations have not been made at present to determine the relative susceptibility of these varieties. It is evident from the amount of this rot found in various samples of fruit from all the cranberry-growing sections the past two or three years that it is the cause of much loss. Whether the recent increase of this rot indicates that the disease is becoming more serious or has only appeared more frequently the past year or two on account of unusually favorable conditions for its development and distribution is not known.

[1] Grateful acknowledgment is due Miss A. M. Beckwith and Mr. Bert A. Rudolph, of the Bureau of Plant Industry, for assistance in making cultures and sections of the fungus, and to Dr. H. J. Franklin, of the Massachusetts State Cranberry Experiment Station, who carried out the spraying experiments in Massachusetts.

CHARACTERISTICS OF THE DISEASE

In most cases in which the endrot organism has been isolated from berries, the fruit first showed signs of rot at the blossom end. Plate A, figure 1, shows the characteristic appearance of Late Howe berries in which the rot started at the blossom end. This was so generally the case in the early collections that the name "blossom end rot" was suggested by Dr. Franklin. Later a quantity of rotten fruit was received from Mr. O. G. Malde, of the Wisconsin Cranberry Station at Grand Rapids, Wis. These berries were affected with what he called "stem end rot," because it usually first appeared at the stem end of the berry. Isolations from this lot of fruit gave almost entirely the endrot fungus. Typical cases of the endrot beginning at the stem end are shown in Plate A, figures 2, 3, 4, and 5. Further studies have shown that rot caused by this organism usually begins at one end or the other of the fruit and rarely, if ever, at the side. The common name "endrot" has therefore been adopted to distinguish this disease in a general way from scald, anthracnose, and other rots which usually begin on some other part of the berry. Unfortunately most of the rots of the cranberry are difficult or impossible to distinguish by the superficial appearance of the diseased fruit.

Endrot first appears as a softening of the tissues accompanied by a slightly yellowish or brownish yellow watery discoloration of the skin. The diseased part is lighter colored than the sound portion of the berry, the various colors and shades usually occurring as shown in Plate A. This discoloration spreads as the rot develops, until the whole fruit is involved and becomes soft and elastic to the touch, but remains turgid.

CAUSAL ORGANISM

The tissues of the fruit affected with endrot are found to contain numerous hyalin, branched fungus filaments which when transferred to favorable culture media produce the characteristic growth and fructifications of one of the Sphaeropsidaceae which appears to be undescribed. In the present state of knowledge of this group of fungi it is difficult to assign the organism satisfactorily to a particular genus. For the present, or until its full life history is positively determined, it may be referred to the genus Fusicoccum as *F. putrefaciens*, n. sp., and characterized as follows:

Fusicoccum putrefaciens, n. sp.

Pycnidia subglobose to pyriform, rather thick-walled, more or less roughened, tawny to tawny brownish, embedded at first, becoming erumpent or subsuperficial when mature, sessile, or subsessile, simple or irregularly chambered forms 300 to 400 μ in diameter, larger chambered forms 400 to 450 μ; pycnospores elliptic to fusiform, hyalin or very faintly yellowish in mass, continuous or pseudoseptate, 8 to 18 by 2 to 3 μ, mostly 10 to 12 by 2.5 μ; sporophores simple or branched, cylindrical or somewhat tapering above, 20 to 36 by 2 μ.

Type specimen.—No. 2918 on rotten prolific cranberries grown near Walton Junction, Michigan, 1916.

Distribution.—Maine to New Jersey and west to Oregon and Washington.

The pycnidia in culture are ovoid to globose or depressed globose with simple or irregular labyrinthiform chambers, erumpent or subsuperficial, 500 to 800 μ in diameter. The inner wall of the pycnidium is sordid yellowish and the pycnospores somewhat more variable in their extreme measurements than in specimens found on old cranberries, under natural conditions, but averaging about the same size. Figure 1 shows sections of pycnidia and spores from a pure culture of the fungus. When mature, the apical portion of the pycnidium ruptures more or less irregularly, permitting the escape of the pycnospores which are expelled in a mucilaginous whitish mass. Later more or less of the top breaks away as shown in figure 2, *a*.

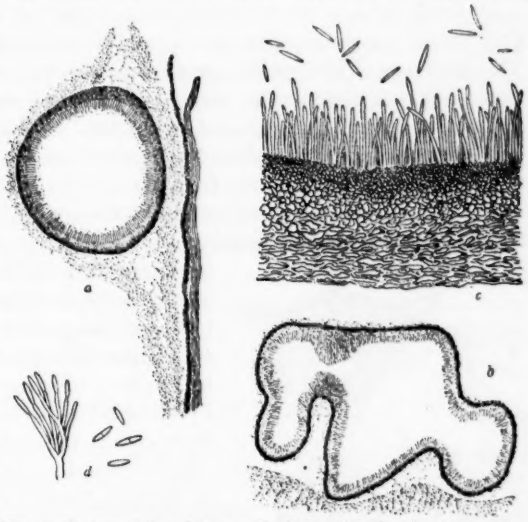

FIG. 1.—*Fusicoccum putrefaciens: a*, Vertical section of a simple pycnidium grown in pure culture on sterile sweet clover stems (*Melilotus alba*); *b*, vertical section of a chambered pycnidium from a corn-meal-agar culture, × 43.5; *c*, part of a section of *a* showing sporophores and spores; *d*, separate sporophores and spores from pure culture, × 420.

FIG. 2.—*Fusicoccum putrefaciens: a*, Median vertical section of a pycnidium growing in the angle beneath the old calyx lobe of an old rotten cranberry, × 43.5; *b*, similar section of a two-chambered pycnidium, with part of the apical portion gone, × 43.5; *c*, portion of inner wall of a pycnidium, showing simple and branched sporophores with immature spores; *d*, separate pseudoseptate spores, × 420.

The pycnidia appear to be produced but rarely under natural conditions in the field, as they have been found only twice, although thorough search of diseased bogs and all parts of the cranberry plant has frequently been made. In a careful examination of a large number of dried rotten berries from a pile of discarded fruit about a year and a half old at the Massachusetts Cranberry Station, a considerable number of mummied berries were found, having a dirty yellowish color suggestive of that produced by the endrot fungus. On one of these berries four or five pycnidia bearing spores were found hidden beneath the old shriveled calyx lobes. Figure 2 shows sections of two of these pycnidia, *a* the simple form and *b* the

chambered form. Sporophores with immature spores from the same specimen are shown in *c* and mature spores in *d*.

In April, 1917, a box of spoiled cranberries was received from Sioux City, Iowa, where they had been stored in the chaff during the winter. This fruit was of the Prolific variety and was grown near Walton Junction, Mich. A considerable part of the berries was affected with a fungus rot having the external appearance of endrot. Two hundred berries of this kind were selected and cultures made from each by transferring a portion of the pulp from the interior of the diseased berries. Of the 200 cultures made, 157 produced the typical growth of the endrot fungus. A considerable number of the entirely rotten and crushed berries from this lot bore pycnidia on and about the blossom end. These fructifications were larger and thicker-walled than those from the old dry fruit illustrated in figure 2. Figure 3 shows sections of pycnidia from the Michigan berries. In these specimens the pycnidia are at first entirely buried in the tissue of the fruit, as shown in figure 3, *a*. As they develop, they break through the epidermis and finally become erumpent or subsuperficial, as shown in figure 3, *b*. When a portion of the sporogenous tissue of the pycnidium is crushed out so that the individual sporophores are separated, it is found that they are frequently branched as shown in figure 3, *c*. The spores often appear septate. No real septum, however, has been demonstrated, and the lack of uniformity in the division of the protoplasm seems to indicate that they are only pseudoseptate.

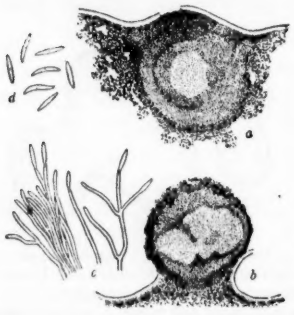

FIG. 3.—*Fusicoccum putrefaciens*: *a*, Vertical section of a young pycnidium embedded in the tissue of a recently rotted cranberry; *b*, vertical section of a nearly mature pycnidium showing two chambers, × 43.5; *c*, separate branched and simple sporophores with immature spores; *d*, separate spores, showing variations in form and size, × 420.

LIFE HISTORY OF THE FUNGUS

Only pycnospores of the form described and illustrated have thus far been produced in cultures. On old rotten, dried, and mummied berries from 1 to 1½ years old, which had been apparently destroyed by the endrot fungus and had been lying on the ground exposed to the weather during the preceding winter, a discomycetous fungus has been found which is suspected of being the perfect form of this plant. The circumstantial evidence is as follows: The apothecia are found on and about the blossom end of the mummied berries which have the peculiar dirty-yellow color characteristic of the endrot fungus. They are also associated with old or obsolete pycnidia of *Fusicoccum putrefaciens*. In section they show the same character and color of the mycelium at the

base and the same relation to the tissues of the host. Unfortunately as yet no cultures from ascospores have been obtained, as all the specimens collected have been too old or poorly developed. This fungus agrees very closely with *Cenangium urceolatum* Ellis (1, p. 9)[1] found on twigs of *Clethra alnifolia* in New Jersey and distributed in Fungi Columbiani No. 742 and North American Fungi Exsiccati No. 990. The author states (1, p. 9) that *Sphaeronema clethrincola* Ellis is the stylosporous form of this species. This statement is apparently based on the close association of the two forms on the same specimens, which may easily be misleading. The writer's suggestion made above in regard to the relation of the endrot fungus to this Discomycete may, however, have but little more value than that of Ellis. It is recorded here for the purpose of calling attention to this fungus, and perhaps thereby securing fresh material with viable ascospores from which pure culture studies may be made, and its life history positively determined.

CULTURAL CHARACTERS

The endrot organism grows well on corn-meal agar, potato agar, steamed corn meal, and stems of *Melilotus alba*. It fruits more frequently on stems than on the other media mentioned.

The superficial growth of the fungus is at first pure white, soon becoming pale green-yellow[2] to pale yellow-green, passing through La France pink to Mars orange, as indicated in Plate A, figure 8, which shows the appearance of a culture 39 days old on cranberry agar-gelatin.

On steam-sterilized stems of *Melilotus alba* the growth passes through the same shades during its early development, but finally becomes nopal-red or even garnet-brown in spots, as indicated in Plate A, figures 6 and 7, which show the appearance of cultures 46 days old.

On corn-meal agar in petri dishes colonies 12 days old observed by transmitted light are white at the margin, passing through pale lemon-yellow and lemon-chrome to orange-citrine at the center and when older on slant agar tubes of the same medium produce colors very similar to those shown in Plate A, figure 8.

CONTROL OF THE DISEASE

As already stated, endrot does not usually show much evidence of its presence in the fruit before picking. The time and manner of infection have not yet been positively determined. From the fact that spraying during the growing season greatly reduces the rot, which usually develops after picking, it seems probable that infection occurs before the fruit is mature and that the fungus is in the berries in a dormant or slowly incubating condition when they are picked.

[1] Reference is made by number to "Literature cited," p. 41.

[2] The nomenclature of colors in the description of the cultures is according to Ridgway. (RIDGWAY, ROBERT. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., 53 col. pl. Washington, D. C., 1912.)

Under ordinary commercial conditions of handling after picking, sometimes 25 to 50 per cent of the Late Howe variety, which is one of the most seriously affected, will show endrot when screened and sorted for shipment two or three months after harvesting. In spraying experiments with Bordeaux mixture, carried on for several years in cooperation with the Massachusetts Agricultural Experiment Station at East Wareham, it has been found that in all cases there was a decided improvement in the keeping quality of the sprayed fruit and in some cases two or three times as much endrot developed in the unsprayed fruit as in the sprayed fruit. Details of the results of these experiments are given by the published reports of Franklin (3, 4, 5) and of the writer (6, 7).

The following formula and dates of applications followed in the general treatment of other cranberry rots have been found effective in reducing loss from this disease: Bordeaux mixture (4–3–50) plus 2 pounds of commercial resin-fishoil soap. The first application should be made just before the blossoms open, the second just as soon as most of the fruit is set, the third about 10 days later, and the fourth when the fruit is about three-fourths grown. Even though spraying is practiced, in order to reduce the loss from endrot to the minimum, it is necessary to take all practicable precautions to pick and handle the fruit with care and place it in a cool, well-ventilated place as soon as possible. A low temperature is very essential in preventing the development of this rot in storage, as it has been found to develop to some extent at 0° C. (32° F.).

Whether or not it will be profitable to practice spraying for this disease will depend largely upon the amount of loss from decayed fruit usually sustained in any individual case. The greatly improved keeping and shipping quality of the sprayed fruit should, of course, be given due consideration in this connection.

It may also be mentioned that under the conditions obtaining on the sprayed plots at the Massachusetts State bog, some injury to the vines is apparent and, associated with it except for the second year (2, 5), was a reduction in the amount of fruit produced. The effect on the vines is not a burning of the new foliage as in ordinary injury by Bordeaux mixture, but a reddening of the old foliage. The cause of this is not at present understood. No injury of this sort has been observed in New Jersey, where spraying with Bordeaux mixture has been practiced on the same bogs for 8 or 10 years in succession. Further investigation and experiments in Massachusetts are necessary to determine if possible whether this case is due to some peculiar local condition or combination of conditions of this bog, and if so, what they are. No injury to vines has been reported or observed on other Massachusetts bogs which have been sprayed with this mixture for several years in succession.

SUMMARY

Endrot is a disease of the cranberry caused by a fungus which does not appear to have been described heretofore.

It has been found to occur in all of the cranberry-growing sections of the United States and in the past few years has occasioned considerable loss especially to the Late Howe variety.

The rot usually starts at either the blossom or stem end of the berry, finally producing complete softening of the fruit.

The causal organism is a sphaeropsidaceous fungus which is here described and named "*Fusicoccum putrefaciens*, n. sp."

Only the pycnidial form has been produced in culture. It is suspected on circumstantial evidence of being genetically related to a species of Cenangium resembling *C. urceolatum* Ellis.

Cultures produce a characteristic series of colors in the mycelium, and pycnidia and pycnospores are produced especially on stems of *Melilotus alba*.

Spraying experiments in Massachusetts have shown that this rot can be largely prevented by the use of Bordeaux mixture.

Some injury to the cranberry vines has been associated with the application of Bordeaux mixture on the experimental plots at the Cranberry Experiment Station in Massachusetts, but not elsewhere. The cause is being investigated.

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1913. CRANBERRY SPRAYING EXPERIMENTS IN MASSACHUSETTS IN 1912. *In* Rpt. 26th Ann. Meeting Cape Cod Cranberry Growers' Assoc., 1913, p. 9–14.

PLATE A

1.—Five Late Howe cranberries showing typical appearance of endrot starting at the blossom end.

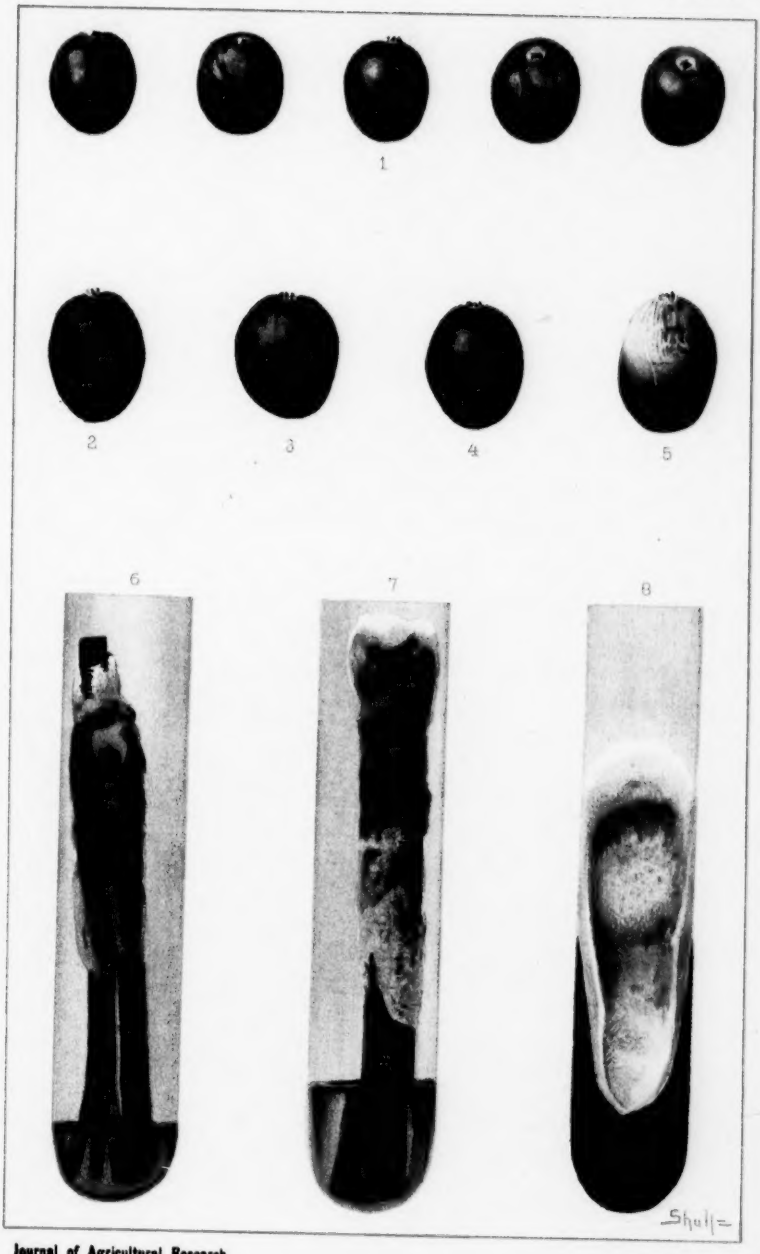
2, 3.—Cranberries from Wisconsin, showing the appearance of the endrot starting at the stem end.

4.—Entirely rotten berry affected with endrot.

5.—Appearance of endrot on the lighter-colored fruit, beginning at the stem end. Cultures of *Fusicoccum putrefaciens* were isolated from the diseased tissues of all these berries.

6, 7.—Cultures of the endrot fungus 46 days old on stems of *Melilotus alba*, showing the various color changes in the fungus from the youngest to the oldest growth.

8.—Culture of the endrot fungus 39 days old on cranberry agar-gelatin, showing the various colors produced.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SOME FACTORS AFFECTING NITRATE-NITROGEN ACCUMULATION IN SOIL

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INTRODUCTION

The literature of nitrification in soils impresses one with the varied technic employed in such studies. It is an exception to find identical methods in use in any two laboratories. In many instances the differences are so slight that they would seem to have little effect upon nitrification, but in other instances the differences are too great, it would seem, not to influence bacterial activity. The variations in volume of soil, depth of column, ratio of volume to surface exposed, shape, size, and type of container, degree of compactness, and methods of preventing contamination and evaporation suggest that the variations which might be produced in bacterial activity could in part explain the wide variations in results often reported from different laboratories.

PRELIMINARY EXPERIMENT

Some preliminary experiments designed to test the validity of such a conception were conducted. No attempt was made to duplicate identically the various methods that have been used or suggested, but the principal variable factors were varied sufficiently to produce different results, provided differences actually arose from such variations. In Table I are reported the variations to which a soil was submitted, together with the results such variations produced upon nitrate accumulation.

In these and most of the succeeding experiments an Oswego silt loam of the following mechanical analysis was used: Fine gravel, none; coarse sand, 0.6 per cent; medium sand, 0.3 per cent; fine sand, 1.5 per cent; very fine sand, 6.9 per cent; silt, 71.8 per cent; clay, 17.8 per cent.

Ammonium sulphate was added at the rate of 60 mgm. of nitrogen per 100 gm. of soil; calcium carbonate, 1 gm. per 100 gm. of soil; water, 27 gm. per 100 gm. of soil. Incubation was for four weeks at room temperature. Nitrate nitrogen, as in all succeeding experiments, was determined by the phenoldisulphonic-acid method as modified by Lipman and Sharp (9). The results in this experiment are reported in milligrams of nitrogen recovered as nitrates per 100 gm. of soil. Ammonia was tested qualitatively by Nessler's reagent. Where reported as "abundant," a heavy red precipitate was formed. Where reported as "some,"

Journal of Agricultural Research,
Washington, D. C.
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Vol. XI, No. 2
Oct. 8, 1917
Key No. Kans.—10

a strong yellow color without precipitate was formed. Where reported as "slight" or "trace" only a very light yellow color developed upon the addition of the reagent.

TABLE I.—*Variations in the nitrates and ammonia produced in Oswego silt loam under different conditions of incubation*

Sample No.	Container.	Quantity of soil.	Nitrogen as NO_3.[a]	Ammonia (NH_3).
		Gm.	*Mgm.*	
1	100-c. c. Erlenmeyer flask, cotton-plugged	50	52.8	Slight.
2	do	100	52.8	Trace.
3	350-c. c. Erlenmeyer flask, cotton-plugged	50	48.4	Slight.
4	do	100	52.8	Trace.
5	do	200	54.2	Do.
6	500-c. c. Erlenmeyer flask, cotton-plugged	50	50.4	Do.
7	do	100	54.0	Do.
8	do	200	54.0	Do.
9	do	400	56.9	Do.
10	1,000-c. c. Erlenmeyer flask, cotton-plugged	50	53.5	Slight.
11	do	100	54.2	Trace.
12	do	200	54.2	Do.
13	do	400	54.8	Do.
14	do	800	55.0	Do.
15	100-c. c. bottle, cotton-plugged	50	54.0	Slight.
16	do	100	54.2	Trace.
17	250-c. c. bottle, cotton-plugged	50	54.0	Slight.
18	do	100	55.0	Trace.
19	do	200	52.8	Do.
20	500-c. c. bottle, cotton-plugged	50	44.9	Abundant.
21	do	100	54.2	Slight.
22A	do	200	53.0	Trace.
22B	do	200	53.5	Do.
23A	do	400	.....	Do.
23B	do	400	54.8	Do.
23C	do	400	52.8	Do.
23D	do	400	52.8	Do.
24X	500-c. c. bottle, cotton-plugged	[a] 100	19.0	Abundant.
24Y	do	[a] 100	52.8	Slight.
25XA	do	[a] 400	9.9	Abundant.
25XB	do	[a] 400	1.8	Do.
25XC	do	[a] 400	Trace.	Do.
25XD	do	[a] 400	Trace.	Do.
25YA	do	[a] 400	44.0	Do.
25YB	do	[a] 400	35.2	Do.
25YC	do	[a] 400	35.2	Do.
25YD	do	[a] 400	35.2	Do.
26	500-c. c. beaker, cotton-plugged	[a] 100	50.0	Slight.
27	500-c. c. beaker, petri-dish cover	100	52.8	Do.
28	Tumbler, cotton-plugged	100	52.8	Do.
29	Tumbler, petri-dish cover	100	52.8	Trace.
30	1-pint fruit jar, cotton-plugged	100	52.8	Do.
31	1-pint fruit jar, petri-dish cover	100	56.9	Do.
32	do	200	55.0	Do.
33	do	500	54.2	Do.
34A	500-c. c. bottle, corked	300	18.2	Abundant.
34B	do	300	18.2	Do.
34C	do	300	18.2	Do.
35	500-c. c. bottle, petri-dish cover	100	55.0	Trace.

[a] The figures reported are averages of closely agreeing duplicates. Where duplicates varied widely, they are both reported as X and Y. A, B, C, etc., have reference to the depth from which samples were taken for analysis. When no letter is given, the whole sample was analyzed or thoroughly mixed and a 100-gm. sample taken. A, is in all cases the surface 100 gm.; B, second 100 gm.; C, third 100 gm., etc.

TABLE I.—*Variations in the nitrates and ammonia produced in Oswego silt loam under different conditions of incubation*—Continued

Sample No.	Container.	Quantity of soil.	Nitrogen as NO_3.	Ammonia (NH_3).
		Gm.	*Mgm.*	
36	Large petri dish, open	100	54.2	Slight.
37	do	[a] 100	54.2	Trace.
38A	20-inch cylinder, cotton-plugged	[a] 900	52.8	Do.
38B	do	[a] 900	58.0	Do.
38C	do	[a] 900	55.0	Do.
38D	do	[a] 900	57.2	Do.
38E	do	[a] 900	58.0	Do.
38F	do	[a] 900	59.8	Do.
38G	do	[a] 900	58.0	Do.
38H	do	[a] 900	55.0	Do.
39A	do	[a] 1,400	52.8	Some.
39B	do	[a] 1,400	49.0	Do.
39C	do	[a] 1,400	37.4	Do.
39D	do	[a] 1,400	26.4	Abundant.
39E	do	[a] 1,400	7.0	Do.
39F	do	[a] 1,400	.9	Do.
39G	do	[a] 1,400	Trace.	Do.
39H [b]	do	[a] 1,400	00.0	Do.
40	500-c. c. bottle, sealed	100	55.0	Trace.
41	Large Fernbach flask	100	55.0	Do.
42	do	1,000	58.3	Do.

[a] Packed. [b] Samples below this depth were not analyzed.

The data recorded in Table I support the following observations:

(*a*) The quantity of soil varied from 50 to 1,000 gm., with but little, if any, effect upon nitrate accumulation. Apparently there were slightly larger quantities of nitrate nitrogen in the large volumes of soil and also slight quantities of ammonia nitrogen in the smaller volumes. However, the differences in both instances are probably within the limit of error. There is no evidence that increasing the volume of soil decreases nitrate formation.

(*b*) The depth of the column of soil varied from ¼ inch (No. 42) to 20 inches (No. 39) without producing any appreciable effect upon nitrate accumulation, provided the soil was left loose. There is also no evidence that nitrate formation 20 inches below the surface was any less vigorous than at the surface, provided the soil was left unpacked.

(*c*) Packing the soil in a thin layer (No. 38) was without effect. If, however, the depth of the column was appreciably increased, packing (reducing volume from 14 to 9) decreased very markedly the nitrate accumulation (No. 40), the accumulation becoming negative only a few inches below the surface. In view of the data to follow, it should be borne in mind that, with a water content of 27 c. c. per 100 gm. of soil, reducing the volume of this soil from 14 to 9 increased the percentage saturation from one-half to practically complete saturation.

(*d*) Decreasing the ratio of surface exposed per 100 gm. of soil from 314 square centimeters (No. 42) to 2 square centimeters (No. 39) was without effect upon the nitrate accumulation.

(*e*) The shape and size of the container, as well as methods of preventing evaporation and contamination, were without effect except when the container was stoppered tightly, and the volume of inclosed air was relatively small in proportion to soil volume.

The principal vital factor which the above variations would seem to affect is the access of oxygen. The fact that the factors influencing the access of oxygen could be varied as widely as in the experiments just recorded without in turn effecting the aerobic processes, suggested the advisability of further study along similar lines. The subsequent experiments herein reported were therefore designed to ascertain the effect produced upon the accumulation of nitrate nitrogen in soils by varying some of the more important factors controlling aeration.

According to Buckingham (1), aeration is a direct function of porosity or free pore space. This being true, the factors influencing porosity are the factors influencing aeration. In any given soil porosity is a direct function of compactness and moisture content, being in an inverse ratio to either one considered alone. In addition to these two variables, it is also essential to take into consideration the length of column or depth to which air or oxygen must diffuse. Accordingly these were the factors varied in the succeeding experiments. Typical experiments of those conducted will now be reported.

EXPERIMENTAL DATA

The data reported in Table II were secured under the following conditions: All samples contained 50 mgm. of nitrogen as ammonium sulphate and 0.5 gm. of calcium carbonate per 100 gm. of soil. Samples were incubated in a moist chamber at room temperaure for the periods indicated in the table. Such loss in moisture as occurred was replaced from time to time. Samples 1 and 2 were contained in large, open petri dishes. Samples 3 and 4 were incubated in wide-mouthed 500-c. c. bottles plugged with cotton. Samples 5 and 6 were in glass cylinders 24 inches high and 2½ inches in diameter. The moisture content of all samples was 20 c. c. per 100 gm. of soil. The samples marked "loose" were placed in the containers and the latter gently tapped on the bottom to cause the soil to settle into the larger openings. Those marked "compact" were so tamped that the same weight of soil occupied only two-thirds the volume that the "loose" soil occupied or in other words there were 1½ times by weight as much soil in same containers.

From the data contained in Table II the following facts seem evident: (1) The "compact" samples in the major portion of possible comparisons show a higher nitrate gain than do the "loose" samples. The greater gains in "loose" samples are so small as to be negligible. (2) The deeper soil columns show higher nitrate gains than do the shallow. (Samples 5 and 6 are exceptions, but for some reason these failed to show active nitrification. Other data recorded in this paper show that this low nitrifica-

tion could not be due to the depth of column. Also, if access of oxygen were influencing nitrification these samples should have shown greater nitrate accumulation in upper layers.) (3) The lower-lying strata of all samples show higher nitrate gains than do the upper layers.

TABLE II.—*Effect of variation in depth of column and compactness of soil upon nitrate accumulation*

[Results are expressed as gain or loss in milligrams of NO_3 per 100 gm. of soil]

Sample No.[a]	Ratio, diameter to depth.	Depth of column.	Sample from depth of—	Incubation.					
				10 days.		20 days.		30 days.	
				Loose.	Compact.	Loose.	Compact.	Loose.	Compact.
		Inches.	*Inches.*						
1.....	14:1	0.4	0–4	0.05	0.60	.....	4.50	12.60	.....
2.....	7.5:1	1.25	0–1.25	−2.00	2.90	6.20	10.20	12.70	26.00
3A.....	3.5:1	2.5	0–1.25	− .22	8.60	7.30	28.80	26.80	36.00
3B.....	3.5:1	2.5	1.25–2.5	.59	5.70	7.10	28.20	27.40	35.50
4A.....	1.75:1	5.0	0–1	4.60	4.50	31.60	32.50	35.60	41.40
4B.....	1.75:1	5.0	2–3	6.62	6.40	31.50	41.50	44.90	51.80
4C.....	1.75:1	5.0	4–5	7.08	4.40	32.60	44.60	47.00	55.90
5A.....	1:2.5	18.0	0–2	.....	.....	.....	.....	− .20	.50
5B.....	1:2.5	18.0	8–10	.....	.....	.....	.....	3.80	3.40
5C.....	.....	18.0	16–18	.....	.....	.....	.....	2.40	10.80
6A.....	1:9	24.0	0–2	.70	−4.30	.....	.....	− .40	4.70
6B.....	1:9	24.0	7–9	− .20	− .50	.....	.....	3.80	3.50
6C.....	1:9	24.0	10–12	1.80	1.80	.....	.....	.....	.....
6D.....	1:9	24.0	14–16	− .80	.20	.....	.....	10.80	23.40
6E.....	1:9	24.0	18–20	− .60	.60	.....	.....	10.70	26.70
6F.....	1:9	24.0	22–24	1.20	2.30	.....	.....	20.20	23.40

[a] A, B, C, etc. are different layers of same column.

The data reported in Table III were secured from samples containing 50 mgm. of nitrogen as ammonium sulphate and 0.5 gm. of calcium carbonate per 100 gm. of soil. They were incubated at room temperature for three weeks. Water lost by evaporation was replaced from time to time. Soil was incubated in 500-c. c. wide-mouthed bottles. The water content varied as indicated in Table III. The samples marked "loose" were as in previous experiments. Those marked "medium" were tamped to two-thirds the "loose" volume. Those marked "compact" were packed to four-sevenths the original volume. It was difficult to reduce further the volume of this soil. In some of the following experiments it was possible by continuous pounding to reduce some samples to one-half the "loose" volume, but this was exceedingly difficult either with high or low moisture contents. With low-water contents such tightly packed samples would burst the heavy glass cylinders upon slight change in temperature. The above phenomenon, together with the fact that an unprotected column of such packed soil 6 inches long would support considerable stress applied at right angle to the length without breaking, gives some idea of how tightly the soil in this and succeeding experiments was packed.

TABLE III.—*Effect of variations in depths of column, moisture content, and compactness of a soil upon nitrate accumulation*

[Results are expressed as gain or loss in milligrams of NO_3 per 100 gm. of soil]

Sample No.[a]	Depth of column.	Sample from depth of—	Water per 100 gm. of soil.	Loose.	Medium.	Compact.
	Inches.	*Inches.*	*C. c.*			
1A	5	0–1	15	0.34	0.84	13.40
1B	5	4–5	15	2.36	6.50	10.70
2A	5	0–1	20	7.80	21.40	.....
2B	5	4–5	20	6.30	35.70	31.60
3A	5	0–1	30	76.50	25.80	−10.70
3B	5	4–5	30	123.80	− 7.60	[b]−14.30
4A	5	0–1	38	185.70	− 8.20	[b]−14.30
4B	5	4–5	38	114.80	[b]−14.30	[b]−14.30

[a] A and B are different layers of same column. [b] Complete loss of NO_3 initially present.

Here again the evidence shows that with a water content of 15 c. c. per 100 gm. of soil the nitrate accumulation increased with an increasing compactness and also with an increasing depth of column. With a moisture content of 20 c. c. per 100 gm. of soil the medium and compact samples both showed a greater nitrate gain than the loose. There was, however, slight difference between the two. Again, there is no evidence of lack of aeration in the lower-lying strata, the medium compact sample showing a much higher gain at the bottom than at the surface.

At moisture contents of 30 and 38 c. c. per 100 gm. of soil, compacting inhibited nitrate accumulation in all except the surface of medium compact samples containing 30 c. c. of water. However, at both these moisture contents the compact samples were saturated. The medium samples were saturated at 38 c. c. and approached very closely to saturation at 30 c. c. per 100 gm. of soil; hence, nitrification could not be expected to take place in them. At 30 c. c. of moisture per 100 gm. of soil the loose samples showed marked increases in the lower layers over the surface layers, while at 38 c. c., which is slightly above the optimum moisture content for this soil in loose condition, the surface soil showed the largest net gain.

In the experiments reported in Table IV combinations of the three variable factors are presented. The calcium-carbonate content was the same as in previous experiments, while the nitrogen added was only 20 mgm. per 100 gm. of soil. The samples were contained in 500-c. c. bottles and tall glass cylinders, and were incubated in a moisture chamber at room temperature for three weeks. In the samples marked "medium" the volume was reduced to three-fourths that of the "loose," while the samples marked "compact" were reduced to one-half that of the "loose." The ammonia was tested qualitatively in order to show whether or not nitrification was complete.

TABLE IV.—*Effect of variations in depth of column, moisture content, and compactness upon nitrate accumulation*

[Results are expressed as gain or loss in milligrams of NO_3 per 100 gm. of soil]

Sample No.[a]	Depth column.	Sample from depth of—	12 c. c. of water per 100 gm. of soil.						24 c. c. of water per 100 gm. of soil.						36 c. c. of water per 100 gm. of soil.[c]			
			Loose.		Medium.		Compact.		Loose.		Medium.		Compact.		Loose.		Medium.	
			NO_3	[b] NH_3	NO_3	[b] NH_3	NO_3	[b] NH_3	NO_3	[b] NH_3	NO_3	[b] NH_3	NO_3	[b] NH_3	NO_3	[b] NH_3	NO_3	[b] NH_3
	Inches.	*Inches.*																
1A	2	0– 2	13.0	Ab	22.0	Ab	16.4	Ab	73.0	T	88.0	T	−1.0	Ab	68.0	T	24.6	Ab
2A	4	0– 2	19.8	Ab	22.0	Ab	11.3	Ab	88.0	T	58.0	T	−2.0	Ab	68.0	T	2.0	Ab
2B	4	2– 4	28.0	Ab	22.0	Ab	16.4	Ab	78.0	T	44.0	Ab	−1.3	Ab	73.0	T	−0.2	Ab
3A	8	0– 2	28.0	Ab	28.0	Ab	16.4	Ab	88.0	S	−2.0	Ab	−2.0	Ab	73.0	T	−0.6	Ab
3B	8	2– 4	26.0	Ab	28.0	Ab	19.8	Ab	88.0	T	−2.0	Ab	−2.0	Ab	73.0	T	2.0	Ab
3C	8	4– 6	.....	.....	32.4	Ab	.....	.....	.....	.....	−2.0	Ab	X	X	.....	.....	X	X
3D	8	6– 8	28.0	Ab	30.0	Ab	24.6	Ab	88.0	T	−2.0	Ab	−2.0	Ab	73.0	T	X	X
4A	16	0– 1	32.4	Ab	16.4	Ab	14.3	Ab	70.0	S	98.0	T	22.0	Ab	84.0	T	6.0	Ab
4B	16	1– 2	.....	.....	22.0	Ab	15.2	Ab	.....	.....	84.0	T	−0.8	Ab	.....	.....	−2.0	Ab
4C	16	2– 3	.....	.....	28.0	Ab	15.2	Ab	.....	.....	78.0	T	−2.0	Ab	.....	.....	X	X
4D	16	3– 4	.....	.....	28.0	Ab	.....	.....	.....	.....	84.0	T	.....	.....	.....	.....	X	X
4E	16	4– 5	.....	.....	.....	Ab	18.4	Ab	.....	.....	78.0	T	X	X	.....	.....	X	X
4F	16	5– 6	.....	.....	28.0	Ab	.....	.....	.....	.....	84.0	T	.....	.....	.....	.....	X	X
4G	16	6– 7	.....	.....	38.0	Ab	.....	.....	.....	.....	73.0	T	.....	.....	.....	.....	X	X
4H	16	7– 9	28.0	Ab	32.4	Ab	22.0	Ab	78.0	T	84.0	T	X	X	78.0	T	X	X
4I	16	9–10	.....	.....	32.4	Ab	.....	.....	.....	.....	84.0	T	.....	.....	.....	.....	X	X
4J	16	10–11	.....	.....	34.8	Ab	.....	.....	.....	.....	84.0	T	.....	.....	.....	.....	X	X
4K	16	11–12	.....	.....	46.0	Ab	.....	.....	.....	.....	78.0	T	.....	.....	.....	.....	X	X
4L	16	12–13	.....	.....	32.4	Ab	22.0	Ab	.....	.....	84.0	T	X	X	.....	.....	X	X
4M	16	13–14	.....	.....	46.0	Ab	.....	.....	.....	.....	84.0	T	.....	.....	.....	.....	X	X
4N	16	14–15	.....	.....	38.0	Ab	.....	.....	.....	.....	98.0	T	.....	.....	.....	.....	X	X
4O	16	15–16	32.4	Ab	41.6	Ab	22.0	Ab	82.0	T	73.0	T	X	X	73.0	T	X	X

[a] A, B, C, etc. are successive layers of the same column.
[b] Ab=NH_3 abundant; S=some NH_3; T=NH_3 trace; X=sample not analyzed, because sample above showed complete loss of NO
[c] Compact samples not set up.

TABLE V.—*Effect of variations in depth in column, moisture content, and compactness upon nitrate accumulation*

[Results are expressed as gain or loss in milligrams of NO_3 per 100 gm. of soil]

Sample No.[b]	Depth of column.	Sample from depth of—	10 c. c. of water per 100 gm. of soil.[a]				15 c. c. of water per 100 gm. of soil.						20 c. c. of water per 100 gm. of soil.						25 c. c. of water per 100 gm. of soil.					
			Loose.		Medium.		Loose.		Medium.		Compact.		Loose.		Medium.		Compact.		Loose.		Medium.		Compact.	
			NO_3.	cNH_3.	NO_3.	cNH_3.	NO_3.	cNH_3.	NO_3.	cNH_3.	NO_3.	cNH_3.	NO_3.	cNH_3.	NO_3.	cNH_3.	NO_3.	cNH_3.	NO_3.	cNH_3.	NO_3.	cNH_3.	NO_3.	cNH_3.
	Inches.	*Inches.*																						
A	13	0–1.5	0.6	Ab	1.4	Ab	13.4	Ab	13.0	Ab	5.8	Ab	54.4	Ab	62.2	Ab	43.0	Ab	98.0	Ab	122.0	Ab	43.0	Ab
B	13	1.5–3									8.0	Ab					48.0	Ab					20.5	Ab
C	13	3–4.5			0.6	Ab			19.2	Ab	10.0	Ab			88.0	T	48.0	Ab			126.4	S	0.0	Ab
D	13	4.5–6									10.4	Ab					40.9	Ab					0.0	Ab
E	13	6–7	1.0	Ab	.06	Ab	28.0	Ab	23.8	Ab	10.4	Ab	70.0	T	76.0	T	34.0	Ab	118.0	T	131.3	S	0.0	Ab
F	13	7–8.5									13.6	Ab					30.1	Ab					0.0	Ab
G	13	8.5–10			0.6	Ab			21.4	Ab	16.0	Ab			79.6	T	30.1	Ab			131.3	S	0.0	Ab
H	13	10–11.5									16.0	Ab					24.4	Ab					0.0	Ab
I	13	11.5–13	1.0	Ab	0.6	Ab	33.0	Ab	22.5	Ab	18.0	Ab	79.6	T	76.0	T	24.4	Ab	136.0	T	126.4	Ab	0.0	Ab

[a] Compact not set up.

[b] A, B, C, etc.=different layers of the same column.

[c] Ab=abundant; S=some; T=trace.

With a moisture content of 12 c. c. per 100 gm. of soil the medium packed gave a greater nitrate accumulation than the loose. The compact, however, showed a somewhat smaller accumulation. In all three instances increasing the depth of column increased the nitrate accumulation, indicating no lack of aeration in the lower layers. Even at 16 inches below the surface in the compact sample the nitrate content was much greater than at the surface.

With a moisture content of 24 c. c. per 100 gm. of soil, the nitrification of the added nitrogen was complete, both in loose and medium-packed soils, except samples 2 and 3 of the medium. These samples probably became puddled on the surface in the course of preparation. The compact samples at this moisture content were saturated; hence, they showed no nitrate accumulation except at the surface of No. 4.

At a moisture content of 36 c. c. per 100 gm. of soil, the nitrification was complete in loose samples at all depths. The medium-packed samples were saturated at this moisture content, and only slight nitrification took place, even in surface soil. It was impossible to set up the compact sample with this high moisture content.

The very compact samples 3 and 4, containing 24 c. c. of water per 100 gm. of soil, and the medium compact samples 2, 3, and 4, containing 36 c. c. water per 100 gm. of soil, all showed heavy gas formation, which resulted in the breaking and pushing upward of the columns at different places, the former two near the bottom, the latter three near the top. It is evident from this that there was little or no gaseous exchange even at the high gas pressure necessary to move the tightly packed columns of soil. Water stood on top of the medium-packed samples 2, 3, and 4.

In Table V are reported other experiments in which the moisture content and degree of compactness were varied. These samples were contained in glass cylinders and incubated in moist chamber at room temperature for 16 days. The period was reduced in order that an analysis might be made before nitrification was complete. Nitrification was so active, however, that even in this short period it was complete in many instances. To all samples 0.5 gm. of calcium carbonate and approximately 25 mgm. of nitrogen as ammonium sulphate were added per 100 gm. of soil.

The quantity of nitrate nitrogen found in those samples containing 10 c. c. of water per 100 gm. of soil was too small to be of any significance. With a moisture content of 15 c. c. per 100 gm. of soil, compacting slightly decreased the nitrate accumulation; however, in all three degrees of compactness the nitrate content increased with increasing depth of column. With 20 c. c. of water per 100 gm. of soil, the nitrification was complete in the lower layers of both loose and medium packed, while there was an abundance of ammonia in the surface soil of both. In the compact sample there was a maximum nitrification at 2 to 4 inches deep, below which the accumulation decreased with the increasing depth.

When the moisture content was increased to 25 c. c. per 100 gm. of soil, the total nitrate accumulation in the loose and medium samples was greatly increased, indicating the possibility of less loss of ammonia by volatilization with the higher moisture content. Here again the nitrification was incomplete in the surface soil of both the loose and the medium samples. The nitrate content also increased as the depth increased, except in the lower strata of the medium packed, where the nitrification apparently was not so active. In the compact samples the nitrate accumulation was inhibited except in surface soil. This soil, however, was in a saturated condition.

The evidence thus far submitted seems sufficient to show that increasing the depth of a column of soil, up to at least 2 feet, increases the formation or rather the accumulation of nitrate nitrogen from ammonium sulphate, provided the moisture content is not so high that aeration is prevented. In the type of soil under study aeration will be sufficient for optimum nitrification, at least up to two-thirds the total amount of water the soil will retain with any degree of compactness tried. Packing, of course, decreases the water-holding power almost in the same proportion as the volume of soil is decreased; hence, the quantity of water necessary to limit aeration is correspondingly less. In as compact condition as it was possible to obtain by the methods used and with a moisture content at least up to 15 c. c. per 100 gm. of soil, nitrification was more active a foot below than nearer the surface. With 20 c. c. of moisture per 100 gm. of soil the nitrate accumulation a foot below the surface on very compact soil was far in excess of any nitrification ever observed under field conditions. Compacting this soil type to two-thirds its loose volume increased the nitrate accumulation with any moisture content up to 25 c. c. per 100 gm. of soil.

From the data available one can estimate roughly the free pore space necessary to supply oxygen in sufficient quantities for maximum nitrate accumulation at the depths to which these experiments were carried out. They have not been conducted to greater depths because under our field conditions practically all the nitrate formation takes place in the first foot of soil. One hundred gm. of this soil in loose condition occupies approximately 116 c. c. of space. The specific gravity of this soil is 2.4. Therefore 100 gm. of soil actually occupy approximately 41 c. c., thus leaving 75 c. c. of space to be occupied by water and air. From Tables III and IV it appears that 36 c. c. of water (which is two-thirds that the soil will retain) is approximately the highest moisture content permitting sufficient aeration for optimum nitrification in loose condition. Thirty-eight c. c. did not permit sufficient aeration, while 36 c. c. permitted complete nitrification of added nitrogen. Accordingly 39 c. c., or 33.6 per cent, of pore space were required for optimum aeration.

On applying similar calculations to the medium-packed samples, 100 gm. of soil occupied approximately 80 c. c. of space. The actual volume

of soil is, of course, the same as in the loose condition—that is, 41 c. c. This leaves 39 c. c. to be occupied by air and moisture. From Tables IV and V it appears that 25 c. c. of water gave approximately optimum moisture conditions for nitrification. This leaves 14 c. c., or 17.5 per cent, of free pore space necessary for efficient aeration. Similarly 100 gm. of compact soil occupied approximately 60 c. c. of space. Subtracting the 41 c. c. of soil volume leaves 19 c. c. for air and water. On referring to Table V it is observed that 20 c. c. of water per 100 gm. of soil did not permit sufficient aeration in the lower strata, while 15 c. c. did. Therefore, with an air content of 4 c. c. per 100 gm. of soil, or 6.33 per cent of free pore space, oxygen was supplied in sufficient quantities for maximum nitrification at a depth of 12 inches. Stoklasa (13) says that if the free pore space falls below 2 per cent anaerobic processes set in. Even though these figures are meager and only approximations, it is evident that increasing the moisture content of a soil not only decreases aeration by actually decreasing pore space, but also increases the demands for oxygen, and, hence, the free pore space necessary to supply sufficient oxygen for maximum aerobic processes. This is probably due to a number of factors. According to Rahn (10), the soil-moisture film should be 10 to 20 μ thick for optimum aerobic conditions. Therefore, increasing the moisture content until this thickness is reached should increase aerobic activity, provided saturation does not exclude aeration. Rahn's views are in accord with daily observations that increasing moisture up to a certain point increases bacterial activity. In the soil under study saturation will result before this optimum film thickness is reached. One would expect, therefore, that the more moisture added until saturation inhibits aeration, the greater the aerobic activity will be, and consequently the greater the demands for oxygen. The writers observed that increasing the moisture content of a similar soil in loose condition from 12 to 20 c. c. per 100 gm. of soil, or from 20 to 33 per cent of saturation, increased nitrate accumulation 4.8 times. Increasing the same from 20 to 30 c. c., or from 33 to 50 per cent of saturation, increased NO_3 accumulation 2.6 times that which occurred at 20 c. c. A further increase of from 30 to 42 c. c., or from 50 to 70 per cent, increased the nitrate accumulation 1.4 times. There is no reason for not believing that other aerobic processes are increased in similar proportions.

Then, too, increasing moisture content decreases aeration at a greater rate than it does porosity; especially is this true with relative high moisture contents. This is due to the fact that, as the water content increases the thickness of the films, the continuous air tubes upon which rapid exchange of gases is dependent are closed at the narrow points much faster than they are actually filled with water. When all such passages are closed and all available oxygen consumed, aeration will be inhibited regardless of the free pore space remaining.

The writers can offer no satisfactory reason as to why compacting increases the nitrate accumulation, but suggest the explanation that bringing the soil particles into closer contact increases the thickness of the moisture films by pressing the moisture out of those areas actually in contact, and thus reducing the area to be covered by film. Also by transforming the films surrounding individual soil grains into films surrounding clusters the area to be covered by the film is decreased. The same factors operating in increasing nitrification in compact soil probably operate in increasing nitrification in lower layers. Nitrates accumulate faster in the lower layers of a column, even if evaporation from the surface be stopped.

The question will very naturally arise as to whether the oxygen utilized in the lower depths of such samples as are here reported actually diffused there, or was not already contained in the freshly aerated soil. From the data given above it is very easy to calculate the maximum amount of nitrogen that could be oxidized to NO_3 condition, provided all the inclosed oxygen was utilized in this process. If the soil is saturated, only that oxygen actually dissolved in the water or adsorbed by the soil itself would be available. As is well known, this is insufficient for a measurable accumulation of nitrate nitrogen; and it can therefore be eliminated from consideration. In the very compact soil, with a moisture content of 15 c. c. per 100 gm. of soil, the air content could not exceed approximately 5 c. c., the oxygen content of which could not form more than approximately 1.85 mgm. of NO_3. In Tables IV and V it will be observed that under such conditions the NO_3 formation was more than 10 times this amount. With 20 c. c., in which there must have been a negligible quantity of air, the NO_3 accumulation was in some instances more than 20 times that calculated for 15 c. c. of moisture. Similar calculations can be made for the other degrees of compactness, and in all instances it will be found that the NO_3 formed was many times in excess of that possible from the inclosed oxygen.

The above calculations have been verified experimentally, in that controls have been run with each experiment to show that the nitrate gains could not be due to the oxygen contained in the soil when the experiment was set up. The results reported in Table VI are typical.

Here is given, in parallel columns, the quantity of NO_3 accumulation in open and stoppered bottles when both were filled with soil. With low moisture content and loose conditions nitrification, to a limited extent, is possible in absence of aeration. However, with a very compact soil even containing low quantities of water or with a high moisture content even in loose condition, the quantity of inclosed oxygen is insufficient to prevent decreases in NO_3. In no instance does the quantity formed under inclosed conditions equal that when aeration was possible.

TABLE VI.—*Nitrate accumulation in soil in a sealed container compared with soil with its surface exposed to the atmosphere*

[Results expressed as gain or loss in milligrams of NO_3 per 100 gm. of soil]

Water per 100 gm. of soil.	Condition.	Gain or loss.	
		Open bottle.	Stoppered bottle.
C. c.			
10	Loose	0.60	0.25
	Medium	1.40	0.25
	Compact	.....	.....
15	Loose	13.40	0.82
	Medium	13.00	7.00
	Compact	5.80	— 2.00
20	Loose	54.40	10.00
	Medium	62.20	28.00
	Compact	43.00	— 2.00
25	Loose	98.00	2.50
	Medium	122.00	— 2.00
	Compact	43.00	.....
28	Loose	.....	—14.40
	Medium	.....	—14.40
	Compact	.....	.....

It is evident, therefore, that the nitrate accumulation herein reported was possible only through the atmospheric oxygen diffusing into those depths wherein such accumulations have occurred. The results above reported were somewhat surprising, and it was deemed advidable to attempt a somewhat similar study of undisturbed soil columns, it being possible that the manipulations through which the soil was carried might facilitate aeration even after such vigorous repacking. Accordingly columns were taken of the same soil as used above to a depth of 12 inches and incubated in glass cylinders at room temperature, as indicated in Table VII. This soil had been thoroughly cultivated the previous season to a depth of 7 inches.

TABLE VII.—*Nitrate accumulation in unbroken soil columns as compared with broken columns*

[Results expressed as gain or loss in milligrams of NO_3 per 100 gm. of soil]

Sample No.	Condition.	Water.		Sample from depth of—	Gain or loss.
		Initial.	Final.		
				Inches.	
1	Natural position, unbroken	28	26	Surface	25.65
				8	5.78
				12	— 1.38
2	Inverted, unbroken	28	26	Surface	3.57
				8	— 0.11
				12	— 2.64
3	Natural position, sieved	28	24	Surface	11.09
				8	4.56
				12	2.63
4	Inverted, sieved	28	22	Surface	2.30
				8	8.65
				12	12.33

It will be observed that nitrification was much more vigorous in unbroken soil in natural position down to a depth of 8 inches than in the sieved soil. Below this depth there was a decrease, indicating insufficient aeration. This did not occur in sieved soil. It should be remembered, though, that this soil at such a depth has never been cultivated and also that the percentage of organic matter is low and the percentage of clay high. These factors, coupled with the fact that the soil contained 28 c. c. of water per 100 gm. of soil, produced practically saturated conditions in the lower layers. When the column was inverted, air could not penetrate the lower layers in sufficient quantity to prevent denitrification a few inches below the surface. It will also be observed that the sieved soil column when inverted gave a higher nitrate accumulation than it did in its normal position. All these facts are in accord with the experiments already given.

In addition to the experiments just mentioned, others have been carried out upon a soil that has not been cultivated for seven years. Columns of soil 5 inches in diameter and 7 inches long were taken by driving a sharp edged cylinder into the soil. These were easily transferred, without even cracking, to anaerobic jars through which a current of moist air could be drawn. At the same time 100 gm. samples of the same soil were incubated under as favorable conditions as possible. The quantities of nitrate nitrogen accumulating under such conditions are, of course, small, because of the absence of added nitrogen. Nevertheless the gains were greater in the unbroken column in every instance.

In addition, in order to see whether oxygen could diffuse through such soil columns in excess of that required by the normal activities of the organisms in the column, the following experiments were planned and carried out. Below an unbroken column 5 inches long were placed 2 inches of sieved soil containing ammonium sulphate and calcium carbonate. The column was then pressed down upon the loose soil and the space surrounding it filled with melted paraffin.[1] This ran into all cracks, etc., made in transferring the column from field to jar, thus making conditions such that all oxygen reaching the loose soil below had to pass through the column. Another 7-inch-long column was well moistened over the bottom and lower sides with a solution of ammonium sulphate and then dusted with calcium carbonate. The column was then placed in a jar and melted paraffin poured around to fill all space at bottom and sides. In this instance there was no method of controlling the concentration of ammonium sulphate in the outer layers of soil, and it is possible that it was sufficient to retard nitrification. In both instances the gain in nitrate was far in excess of that in untreated

[1] Since these experiments were carried out, the senior writer has called attention to the danger of the use of paraffin in such experiments. (GAINEY, P. L. EFFECT OF PARAFFIN ON THE ACCUMULATION OF AMMONIA AND NITRATES IN THE SOIL. *In* Jour. Agr. Research, v. 10, no. 7, p. 355-364. Literature cited, p. 363-364. 1917.) In these experiments, however, the paraffin would probably tend to eliminate rather than to accentuate the differences to which attention is called.

soil as well as in excess of any accumulations observed under field conditions.

The moisture content per 100 gm. of soil, incubation period, etc., are given below, along with results reported as milligrams of NO_3 per 100 gm. of soil gained or lost. A sample containing the same quantities of ammonium sulphate and calcium carbonate was also incubated in tightly stoppered bottles, to simulate conditions of loose soil below the unbroken column, provided no oxygen diffused through.

TABLE VIII.—*Nitrate accumulation in unbroken and sieved columns of soil*

[Results expressed as gain or loss in milligrams of NO_3 per 100 gm. of soil]

Sample No.	Depth of column.	Condition.	Moisture content.	Gain or loss.
	Inches.		*C. c.*	
1	7	Unbroken. Incubated 7 weeks	25	9.60
2	7	Sieved, incubated 7 weeks	25	6.50
3	7	Unbroken. Incubated 4 weeks	24	.32
4	7	Sieved, incubated 4 weeks	24	.07
5	5	Unbroken. Loose soil below incubated 4 weeks	24	39.80
6	7	Moistened with ammonium sulphate, inbated 4 weeks.	24	14.50
7	7	Sieved, moistened with ammonium sulphate, incubated 4 weeks.	24	30.00
8	7	Sieved, moistened with ammonium sulphate, sealed, incubated 4 weeks.	24	−.90
9	7	Unbroken. Incubated 12 weeks	24	2.50
10	7	Sieved, incubated 12 weeks	24	1.60

It will be observed that the oxygen contained in the loose soil was insufficient to prevent dentrification, also that oxygen passed to the loose soil below the 5-inch column in sufficient quantity to enable a more rapid accumulation of NO_3 than occurred in loose soil freely exposed to the air. The accumulation in the 7-inch column moistened with ammonium sulphate is also far in excess of that where no nitrogen was added.

DISCUSSION OF RESULTS

The results presented above are certainly sufficient to open the question of whether or not the beneficial effect upon bacterial activity, observed to follow cultivation, can be attributed to increased aeration. The question is not altogether new, for Leather (8) has pointed out that the beneficial effect of cultivation upon crop production must be attributed to some factor other than aeration.

The available data relative to the effect of different degrees of aeration upon nitrate formation is confined almost wholly to the effect that cultivation produces. We do not believe, however, that one is justified in attributing the major effect of cultivation upon nitrification to better aeration. There is no question but that cultivation may beneficially influence nitrate formation other than through increasing aeration. Be

this as it may, it has recently been shown by Call and Sewell (2), of this Station, that just as vigorous nitrate accumulation can take place in noncultivated as in cultivated soil. The following results are taken from their paper. The figures represent pounds of nitrates gained or lost between the given dates. The noncultivated plot was kept bare of weeds by scraping the surface.

Year.	Period.	Not cultivated.	Cultivated 3 inches deep.	Cultivated 6 inches deep.
		Pounds.	*Pounds.*	*Pounds.*
1914	Apr. 5 to Nov. 2	620	84	369
1915	Apr. 15 to Sept. 8	419	180	207
1916	May 29 to Sept. 23	283	281	485
Average		441	182	354

King and Whitson (5) conducted somewhat similar experiments under greenhouse conditions. In one experiment, carried out in the greenhouse, soil in cylinders in which various crops had been grown, was found after 91 days to show an average gain in nitric nitrogen per acre-foot (4,000,000 pounds) in all cultivated soils of 105.62 pounds. Soil in the noncultivated cylinders showed a gain of 162.42 pounds. In another series, which ran for 258 days, the average gain in soil in noncultivated cylinders was 325.48 pounds, and in the soil of all cultivated cylinders, 303.68 pounds per acre-foot. In summarizing they say (p. 9):

> The increase of nitric nitrogen has been greater at all depths, as a rule, where the soils have not been cultivated than where they have been cultivated. As a general rule, there has been the highest increase of nitric nitrogen in the surface foot, and the increase in the third foot has generally exceeded that in the second foot.

King and Whitson (6) also carried out the only experiments found reported wherein an effort was made under field conditions to increase aeration without cultivation. Holes were bored to a depth of 4 feet and left open but protected in order to prevent drying out and also to prevent surface drainage. These were so arranged that other samples could be taken for nitrate analysis at definite distances from the original holes. Duplicate sets were arranged and analyzed both after 4 and 10 weeks' standing. Below are given the average nitrate contents per foot to a depth of 4 feet at the 10-week analysis.

Holes on number of sides	4	4	1	2
Distance from holes, inches	6	8.5	12	24
Nitrates	26.24	23.94	27.00	27.01

If the figures from any depth, even third and fourth foot layers, are studied, there is little to indicate that increasing aeration, as such a procedure must have done, increased the nitrate formation. The moisture

content of this soil varied from about 12 to 25 c. c. per 100 gm. of soil, averaging approximately 20 c. c.

The writers have also endeavored to increase aeration by forcing air daily into horizontal perforated tubes placed underneath both cultivated and uncultivated plots of ground. Such experiments have been conducted for two seasons without the differences in nitrate accumulation exceeding the experimental error.

Such data as are available, therefore, upon the effect of increasing aeration under conditions more nearly approaching normal field conditions are in accord with the laboratory results.

There is another angle from which the question of availability of oxygen in soils of different types, different depths, etc., under natural conditions can be approached. One can readily measure, with a fair degree of accuracy, the oxygen actually potentially available for bacterial metabolism. Such data, together with data as to the quantity of oxygen essential for normal aerobic activity, should be very valuable in connection with the problem under consideration.

Fortunately Schlœsing (12), in his original classic researches upon nitrification, secured very definite data, which, so far as the writers are aware, has never been refuted, on the effect of varying the oxygen content of the atmosphere upon nitrate accumulation. Working with a fertile soil, rich in humus—containing 0.263 per cent of nitrogen and of the following mineral composition: Clay (*argile*), 14.6 per cent; chalk (*calcaire fin*), 19.5; silicious sand (*sable siliceux*), 48; calcareous sand (*sable calcaire*), 17.7—and with a moisture content of 15.9 parts per 100, he secured the results given below:

Oxygen, per cent.....	1.5	6	11	16	21
Nitric acid formed, mgm.....	45.7	95.7	132.5	246.6	162.6

Incubation was for four weeks at temperature of 21° to 29° C.

Duplicating this experiment, except that one sample received no oxygen, the moisture content was 24 parts per 100 of soil (as much as it would absorb), and incubation eight instead of four months, he secured the following results:

Ogygen, per cent.....	0	6	11	16	21
Nitric acid formed, mgm..	−64	199	222	203	225

In this instance, with the capacity of the soil to hold air reduced very low and an oxygen content in the air of only 6 per cent, practically as much nitric acid was formed as with normal atmospheric oxygen content, while with 11 per cent of oxygen the quantity was equal to that of normal air.

It is also interesting to note that the carbon dioxid evolved, a factor, as the evidence shows, more or less dependent upon oxygen content, was practically as great at 6 per cent of oxygen as with 21 per cent.

Below are given the relative averages of carbon dioxid evolved in the two experiments just cited:

Oxygen, per cent	0	1.6	6	11	16	21
Carbon dioxid evolved, mgm. per kgm. of soil in 24 hours	9.03	10.4	16.25	16.05	15.85	17.5

It is evident, therefore, that for either the process of carbon dioxid formation (partially aerobic) or nitric acid formation (obligate aerobic) the oxygen content of the soil atmosphere can be reduced to one-half normal atmospheric oxygen content or below without any appreciable effect upon the processes. Also, the total air content of the soil can be reduced very low without any detrimental effect upon the bacterial activities in question, a fact entirely in accord with the data submitted in this paper. With this information available accurate analysis of soil atmosphere, both as to quantity present and composition, should give us valuable information as to the probability of sufficient quantities of oxygen being available under that particular condition. Fortunately there are a number of recent carefully executed investigations upon soil gases, particularly those of Lau (7), Russell and Appleyard (11), Djarenko (3), Jodidi and Wells (4), and Leather (8).

Lau (7), working with three very different types of soil (sand, loam, and peat) at Rostock, Germany, found, upon analyzing the soil atmosphere monthly for a period of one year, that the carbon-dioxid content varied from 0.11 to 0.143 per cent at a depth of 6 inches; 0.157 to 0.65 per cent at 12 inches; and 0.29 to 2.09 per cent at 24 inches. The oxygen varied from 20.35 to 20.77 per cent at a depth of 6 inches; 19.99 to 20.63 per cent at 12 inches; and 18.42 to 20.19 per cent at 24 inches. Manuring a sandy soil and cropping to potatoes increased the carbon-dioxid from 0.11 to 0.57 per cent and decreased the oxygen content from 20.79 to 20.22 per cent.

Russell and Appleyard (11) analyzed the soil gases under the following conditions at the Rothamstead Station; Broadbalk wilderness (uncultivated); Boradbalk wheat field, both manured and unmanured; and Hoos field, both under wheat and fallow. Analyses were made on an average of three times a month for periods in most cases extending over approximately two years. Below are given the extreme and average results of their analysis when air was drawn from a depth of 6 inches:

Constituent.	Minimum.	Maximum.	Average.
	Per cent.	*Per cent.*	*Per cent.*
Carbon dioxid	0.02 to 0.28	0.38 to 2.27	0.30 to 0.52
Oxygen	17.61 to 20.44	20.81 to 21.30	20.19 to 20.46
Nitrogen	78.49 to 79.01	79.38 to 80.48	79.25 to 79.30

They also found very slight differences in the composition of air drawn from depths of 6 inches and 18 inches

Djarenko (3), investigating the effect of different methods of fallowing practiced in Russia upon the composition of the soil atmosphere, secured the results given below. He analyzed the soil gases 14 times between April 20 and September 1.

	Black fallow.			April fallow.			June fallow.			Peasant fallow.		
	Min.	Max.	Ave.	Min.	Max.	Ave.	Min.	Max.	Ave.	Min.	Max.	Ave.
Oxygen.....per cent..	17.1	20.7	18.9	19.2	21.5	19.9	18.2	20.0	19.4	10.6	19.1	15.5
Air per liter of soil....c. c..	290	410	332	240	420	348	200	330	264	150	280	208
Oxygen per liter of soil.c. c..	50	81.5	64.5	48	84	67.8	36.5	65.9	51.3	16	48	32.7

Black fallow is worked in autumn and again the following April and June. April fallow is worked in April and June. June fallow is first worked in June. Cattle are allowed to roam upon peasant fallow in the spring, thus giving it a much-trodden effect. It is first cultivated in June. All are seeded on August 1.

Jodidi and Wells (4), analyzing soil gases from 22 plots at the Iowa Station daily from April to August, inclusive, found the lowest, highest, and average monthly analyses to be 0.04, 0.82, and 0.25 per cent for carbon dioxid and 19.36, 21.20, and 20.5 per cent, respectively, for oxygen. The 5-month average of individual plots varied from 0.18 to 0.44 per cent for carbon dioxid and 20.18 to 20.62 per cent for oxygen. Samples were drawn from 7 inches below the surface.

Leather (8), in his work upon soil gases at Pusa, India, used very different methods of analysis, which render it difficult to compare his results with those given above. From a definite volume of soil all gases that could be extracted with a Topler pump were removed for analysis. Thus, not only the air existing in free pore space but also much that was held in solution was removed. Owing to the much greater solubility of carbon dioxid than oxygen and nitrogen in water, the percentage present in dissolved air is much higher and as a result the percentage in air extracted from the soil by this method is much higher. Sufficient data are not given for making the necessary corrections in order to place the figures upon the same basis as those already given. By assuming, though, that the five instances in which such corrections are made by the author are representative, the carbon dioxid figures reported below are approximately five times too high, while the oxygen figures should be increased by approximately 15 per cent. According to Leather (8, p. 106)—

> When the total carbon dioxide is much less than 10 per cent and the soil is not particularly dry, it is nearly all in solution.

With these facts in mind are quoted the following figures from Leather's paper. These data were secured from experiments with an unmanured fallow soil and show the variations in analysis for a number of dates.

Depth.	Per cent oxygen.	Per cent CO_2.	Per cent gas in volume.
1st 12 inches	12. 7 to 18. 6	2. 9 to 12	18. 6 to 41. 2
2d 12 inches	9. 2 to 18. 5	3. 9 to 8. 5	14. 8 to 39. 0
3d 12 inches	9. 7 to 17. 7	1. 9 to 6. 9	23. 8 to 42. 2
4th 12 inches	7. 6 to 17. 8	1. 4 to 7. 0	21. 2 to 45. 3
5th 12 inches	11. 9 to 14. 9	2. 7 to 8. 5	3. 8 to 28. 2
6th 12 inches	12. 7 (one analysis).	6.1 (one analysis).	22. 6 to 25. 3
7th 12 inches	15. 5 (one analysis).	8.2 (one analysis).	.....

The low oxygen figures followed the heavy monsoon rains, while the high figures followed prolonged droughts.

As a general summary of the above-reported soil atmospheric analysis, coming from as wide a variety of localities, conditions, etc., as they do, one may say:

(*a*) The percentage of air by volume in soil rarely ever falls below 20 even several feet below the surface where soil has never been artifically disturbed. Excessive rains or excessive trampling of cattle when the soil is moist may reduce the percentage.

(*b*) The percentage of oxygen contained in the free soil atmosphere is normally only slightly below that in the atmospheric air. This is true to such depths as one should expect appreciable nitrification to take place. The exceptional conditions just cited, together with heavy applications of green or stable manure, as well as the presence of certain growing plants, may cause a marked reduction in the oxygen content. Such exceptional reductions rarely ever cause a reduction of more than one-half the atmospheric content, even several feet below the surface.

(*c*) The carbon-dioxid content of the soil air is usually materially higher than that of the atmospheric air. Any factor tending to increase the bacterial activity or to decrease the exchange of gases tends to increase the carbon-dioxid content at the expense of oxygen. The proportion of carbon dioxid present in free soil air rarely ever exceeds 2 per cent, and usually is only a few tenths of 1 per cent. Owing to the greater solubility of carbon dioxid, the dissolved soil atmosphere is usually high in this constituent. According to Leather, the major portion of this is probably in loose chemical combination as an acid carbonate.

Since the composition of the soil atmosphere is directly controlled by the rate of exchange of gases between it and the atmospheric air, it is evident from slight variations in composition reported above that

the exchange of gases between the two must take place very rapidly. This is true under practically all conditions and to such depths for which data are available.

So far as the writers are aware, there are no data available invalidating the assumption that the results reported by Schlœsing (12) are applicable to soils in general. If this be true, it would certainly seem with the experimental data now available, that conditions are rarely ever met with where there is not sufficient oxygen potentially available to insure maximum nitrification. Furthermore, Stoklasa (13) mentions 2 per cent of porosity as the point below which anaerobic processes become active, 3.8 per cent is the lowest found recorded for field conditions, and seldom does it fall below 15 to 20 per cent. The writers have shown that in a tightly compact soil with a moisture content of 15 c. c. per 100 gm. of soil 6.33 per cent of porosity were sufficient for maximum nitrate accumulation at considerable depths below the surface. Therefore these experimental data and theoretical considerations from an entirely different point of view all tend to the same conclusions as do the original experimental data herein reported.

SUMMARY

In the experimental data submitted in this paper it has been shown that:

(1) As the moisture content of a soil decreases, increasing the compactness from a very loose condition will increase the accumulation of nitrate nitrogen.

(2) With any degree of compactness tested the optimum moisture content will be reached when the soil contains approximately two-thirds the total amount of moisture it will retain.

(3) Aeration will be sufficient to the depth of 1 foot with any degree of compactness, provided the moisture content does not exceed the above relation.

(4) Increasing the depth of column up to 2 feet does not, as far as tested, alter the above relations. In fact, the accumulation of nitrate nitrogen increases with increasing depth down to 2 feet, so long as the moisture does not exceed approximately two-thirds saturation.

(5) Nitrate nitrogen accumulates more rapidly in unbroken soil columns than in pulverized soil. Aeration in a column of soil uncultivated for seven years is far in excess of that required to maintain aerobic conditions.

It has also been pointed out that such experimental data as is available, regarding oxygen relations in normal field soils, indicate that obligate aerobic conditions almost universally exists within the first foot of surface. Therefore such beneficial effect as cultivating may have upon biological activity can not be attributed to increased aeration.

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